

WHAT IS CLAIMED IS:

1. A recombinant method for identifying a bioactive peptide comprising:

(a) transforming a host cell comprising Lac repressor protein with an expression vector comprising a tightly regulable control region operably linked to a nucleic acid sequence encoding a peptide, wherein the tightly regulable control region comprises at least a portion of the wild-type *E. coli lac* promoter/operator region, said portion comprising auxiliary *lac* operator O3, a CAP binding region, the -35 *lac* promoter site, the -10 *lac* promoter site, *lac* operator O1, *lacZ* Shine-Dalgarno sequence and a spacer region;

(b) growing the transformed cell under conditions that repress expression of the peptide;

(c) inducing expression of the peptide in the transformed host cell;

(d) determining whether expression of the peptide is inhibitory of host cell growth, wherein inhibition of host cell growth is indicative of the expression of a bioactive peptide.

2. The method of claim 1 wherein the host cell is a bacterium.

3. The method of claim 2 wherein the bacterium is a gram positive bacterium.

4. The method of claim 2 wherein the bacterium is gram negative bacterium.

5. The method of claim 2 wherein the bacterium is *E. coli*.

6. The method of claim 1 wherein the host cell is a microbial pathogen.

7. The method of claim 6 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.

AMENDED SHEET

04-10-2000

US 00992373*

82

8. The method of claim 1 wherein the expression vector comprising the nucleic acid sequence encoding the peptide is a first expression vector, and wherein the host cell is further transformed, prior to step (b), with a second expression vector comprising a promoter operably linked to a gene encoding a Lac repressor protein.
9. The method of claim 1 wherein the expression vector has the identifying characteristics of pLAC11 (ATCC No. 207108).
10. The method of claim 9 wherein the expression vector is pLAC11 (ATCC No. 207108).
11. The method of claim 1 wherein the host cell comprises proteases or peptidases or both.
12. The method of claim 1 wherein the host cell has not been modified to reduce or eliminate the expression of naturally expressed proteases or peptidases.
13. The method of claim 1 wherein the host cell is a prokaryote.
14. The method of claim 1 wherein the host cell is a microbial pathogen.
15. The method of claim 14 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.
16. The method of claim 1 wherein the host cell is a eukaryotic cell.
17. The method of claim 16 wherein the eukaryotic cell is a mammalian cell.
18. The method of claim 16 wherein the eukaryotic cell is a cancer cell.
19. The method of claim 1 wherein the host cell is a protozoan.

AMENDED SHEET

20. The method of claim 1 wherein the peptide comprises a first stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.
21. The method of claim 20 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa.
22. The method of claim 21 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.
23. The method of claim 1 wherein the peptide comprises a stabilizing motif.
24. The method of claim 23 wherein the stabilizing motif comprises a hydrophilic α -helix motif.
25. The method of claim 23 wherein the stabilizing motif comprises an opposite charge ending motif.
26. The method of claim 1 wherein the peptide comprises a randomized amino acid sequence.
27. The method of claim 26 wherein the peptide comprises a first stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.
28. The method of claim 26 wherein the peptide comprises a stabilizing motif.
29. A bioactive peptide comprising a first stabilizing group comprising the N-terminus of the bioactive peptide and a second stabilizing group comprising the C-

AMENDED SHEET

terminus of the bioactive peptide.

30. The bioactive peptide of claim 29 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa.

31. The bioactive peptide of claim 30 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

32. The bioactive peptide of claim 30 wherein the first stabilizing group is Pro-Pro- and the second stabilizing group is -Pro-Pro.

33. The bioactive peptide of claim 29 wherein at least one of the first and second stabilizing groups comprises a small stable protein.

34. The bioactive peptide of claim 33 wherein the small stable protein is a four-helix bundle protein.

35. The bioactive peptide of claim 33 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

36. The bioactive peptide of claim 35 wherein the small stable protein is Rop protein.

37. The bioactive peptide of claim 29 which is an antimicrobial peptide.

38. The bioactive peptide of claim 29 which is a therapeutic peptide drug.

AMENDED SHEET

39. A bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide.

40. A fusion protein comprising a four-helix bundle protein and a polypeptide.

41. The fusion protein of claim 40 wherein the four-helix bundle protein is Rop protein.

42. The fusion protein of claim 41 wherein the polypeptide comprises a bioactive peptide.

43. The fusion protein of claim 41 wherein the four-helix bundle protein is covalently linked at its C-terminus to the N-terminus of the polypeptide.

44. The fusion protein of claim 41 wherein the four-helix bundle protein is covalently linked at its N-terminus to the C-terminus of the polypeptide.

45. A polypeptide comprising:

a bioactive peptide comprising (a) a first stabilizing group selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Xaa-Pro- and -Xaa-Pro-Pro- and (b) a second stabilizing group selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa; and

a cleavage site immediately preceding the first stabilizing group;
wherein the second stabilizing group comprises the C-terminus of the polypeptide.

46. A polypeptide comprising:

a bioactive peptide comprising (a) a first stabilizing group selected from the group consisting of Pro, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- and (b) a second stabilizing group selected from the group consisting of -Pro-, -Pro-Pro-, -Pro-Xaa- and -Pro-Pro-Xaa-; and

AMENDED SHEET

a cleavage site immediately following the second stabilizing group;
wherein the first stabilizing group comprises the N-terminus of the polypeptide.

47. A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and

a cleavage site immediately preceding the plurality of sequential uniformly charged amino acids.

48. A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and

a cleavage site immediately following the plurality of sequential oppositely charged amino acids.

49. A method for using an antimicrobial peptide comprising:

covalently linking a first stabilizing group to the N-terminus of the antimicrobial peptide and a second stabilizing group to the C-terminus of the antimicrobial peptide to yield a stabilized antimicrobial peptide; and

contacting a microbe with the stabilized antimicrobial peptide.

50. The method of claim 49 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.

51. The method of claim 49 wherein the first stabilizing group is selected from the group consisting of Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- and the second

AMENDED SHEET

stabilizing group is selected from the group consisting of -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.

52. A method for using an antimicrobial peptide comprising:

covalently linking a plurality of sequential uniformly charged amino acids to the N-terminus of the antimicrobial peptide and covalently linking a plurality of sequential oppositely charged amino acids to the C-terminus of the antimicrobial peptide to yield a stabilized antimicrobial peptide; and

contacting a microbe with the stabilized antimicrobial peptide.

53. A method for treating a patient having a condition treatable with a peptide drug comprising administering to the patient a stabilized form of the peptide drug.

54. The method of claim 53 wherein the stabilized form of the peptide drug comprises a first stabilizing group comprising the N-terminus of the peptide drug and a second stabilizing group comprising the C-terminus of the peptide drug.

55. The method of claim 54 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.

56. The method of claim 55 wherein the small stable protein is a four-helix bundle protein.

57. The method of claim 55 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione S-transferase, thioredoxin, maltose binding protein and glutathione reductase.

AMENDED SHEET

88

58. The method of claim 54 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking the first stabilizing group to the N-terminus of a peptide drug and covalently linking the second stabilizing group to the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.

59. The method of claim 53 wherein the stabilized form of the peptide drug comprises an opposite charge ending motif.

60. The method of claim 59 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking a plurality of sequential uniformly charged amino acids to the N-terminus of the peptide drug and covalently linking a plurality of sequential oppositely charged amino acids comprising the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.

AMENDED SHEET